

Autoantibody Facilitated Cleavage of C $\bar{1}$ -Inhibitor in Autoimmune Angioedema

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Abstract

C $\bar{1}$ -inhibitor (C $\bar{1}$ -Inh) is an important inhibitor of the inflammatory response and deficiency of this inhibitor, which may be hereditary or acquired, is associated with recurrent episodes of edema. Recently, an autoimmune form of angioedema has been described that is associated with functional deficiency of C $\bar{1}$ -Inh and an autoantibody that impedes C $\bar{1}$ -Inh function. In this report we describe the isolation of C $\bar{1}$ -Inh from the monocytes and plasma of a patient with autoimmune angioedema and demonstrate that the patient's monocytes secrete structurally and functionally normal C $\bar{1}$ -Inh, but show that this protein circulates in the patient's plasma in an inactive, structurally altered form. Furthermore, using analytic gel electrophoresis techniques it is demonstrated that the patient's autoantibody facilitates cleavage of normal C $\bar{1}$ -Inh, by its target proteases, to the same species of C $\bar{1}$ -Inh that is found circulating in the patient's plasma. This autoantibody facilitated cleavage of normal C $\bar{1}$ -Inh is apparently a consequence of destabilization of protease/inhibitor complexes. These findings contribute to our understanding of protease/C $\bar{1}$ -Inh interactions and document important observations on pathogenic mechanisms in autoimmune disease.

Introduction

C $\bar{1}$ -inhibitor (C $\bar{1}$ -Inh)¹ is a heavily glycosylated single chain polypeptide, with a molecular weight of ~ 105 kD, which is synthesized by hepatic and macrophage cells (1, 2). This protein, which circulates in high levels in human plasma, functions as a serine protease inhibitor and inactivates proteases of the contact phase (activated Hageman factor), kinin (kallikrein), coagulation (Factor XIa), complement (C $\bar{1}$, C $\bar{1}$ r, C $\bar{1}$ s), and fibrinolytic (plasmin) pathways (2-4). C $\bar{1}$ -Inh is therefore an important inhibitor of the inflammatory response. Deficiency of C $\bar{1}$ -Inh, which may be inherited or acquired, is associated with a number of biochemically distinct forms of angioedema, all of which are characterized by recurrent attacks of episodic edema typically affecting the extremities, abdomen and face (5, 6). Until relatively recently upper airway obstruction

and sudden death was an outcome that could occur in up to 50% of these patients (7). Although incompletely understood, the attacks of edema appear to be generated by uncontrolled proteins of the complement and kinin pathways possessing potent vasoactive properties (5, 6, 8).

Angioedema associated with C $\bar{1}$ -Inh deficiency most commonly occurs in the clinical setting of an inherited disorder transmitted as an autosomal dominant trait. This hereditary form of angioedema has two separate expressions one of which is associated with absent or very low levels of normally functioning C $\bar{1}$ -Inh and the other with normal or increased levels of a dysfunctional C $\bar{1}$ -Inh protein (5, 6). A rarer form of C $\bar{1}$ -Inh deficiency and angioedema known as acquired angioedema has also been described: this typically affects individuals later in life and is associated with very low levels of antigenic C $\bar{1}$ -Inh (9). In contrast to the hereditary type of angioedema, this disorder is characterized by very low levels of the immunoglobulin binding component of complement, C1q (9). In acquired angioedema, edema is a consequence of enhanced consumption of normally functioning C $\bar{1}$ -Inh, by ongoing complement activation, which is in general secondary to a malignant process.

Recently we described a patient with an unique form of angioedema, autoimmune angioedema, characterized by normal levels of a dysfunctional C $\bar{1}$ -Inh protein and an autoantibody that impedes the function of normal C $\bar{1}$ -Inh (10). In this paper we describe the basic autoimmune process underlying this patient's disorder. It is demonstrated that the patient's monocytes secrete structurally and functionally normal C $\bar{1}$ -Inh but that this protein circulates in plasma in an inactive, structurally modified form. It is established in vitro, that patient autoantibody facilitates cleavage of normal C $\bar{1}$ -Inh by the proteases C $\bar{1}$ r, C $\bar{1}$ s, and plasmin to form a C $\bar{1}$ -Inh species identical to that isolated from the patient's plasma. Furthermore, it is concluded that the autoantibody mediates this effect by destabilizing the acyl intermediate formed during the interaction of normal C $\bar{1}$ -Inh and protease. The high degree of specificity of the patient's autoantibody for normal, uncleaved C $\bar{1}$ -Inh and the structural modifications that occur to C $\bar{1}$ -Inh on interaction with plasmin are documented.

Methods

Case history

The patient, a 63-yr-old man, presented in March 1982 with intermittent attacks of crampy abdominal pain and edema involving the face, hands, and feet. There was no history of similar episodes in the patient or his family. Physical examination revealed nonpruritic, nonpitting edema of one hand but was otherwise negative. Routine laboratory investigation revealed no abnormality. Antigenic serum levels of complement components C1q and C4 were undetectable; C3 and C $\bar{1}$ -Inh were however, present at normal levels. C $\bar{1}$ -Inh functional activity was absent. The patient's family had normal complement profiles. A diagnosis of an acquired functional deficiency of C $\bar{1}$ -Inh was established.

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1. Abbreviations used in this paper: C $\bar{1}$ -Inh, complement 1 inhibitor; DMEM, Dulbecco's minimum essential medium; ZLNE, *N* α -carboxybenzyoxy-*l*-lysine-*p*-nitrophenyl ester.

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Treatment was commenced with the attenuated androgen, danazol, on an initial daily dose of 600 mg tapering to 100 mg over the next 12 mo. All of the patient's complement abnormalities, including C $\bar{1}$ -Inh function, quickly reverted to normal and the patient became and remained symptom free for ~ 1 yr. On reduction of danazol to 100 mg daily, edema recurred and over the following 12 mo the patient gradually became refractory to treatment with danazol, having recurrent attacks of edema of increasing frequency that were resistant to high dose (600 mg/d) therapy with danazol. These attacks of edema were always predated by 1–2 wk by falling serum levels of C4. Continuing clinical and laboratory evaluation failed to reveal any evidence of underlying disease. Frequent attacks (weekly) of severe edema continued to occur. By this time it was apparent that the patient had a circulating IgG autoantibody that impeded normal C $\bar{1}$ -Inh function and a treatment regimen of 30 mg prednisolone daily was commenced. Over the next month a gradual clinical response evolved and the patient became symptom free. However, during three attempts that were made to reduce prednisolone dosage below 10 mg daily, edema recurred and this again settled on a higher treatment schedule. One episode of severe facial edema, occurring while the patient was on a reduced dose of prednisolone, arrested promptly on infusion of 1,000 U of C $\bar{1}$ -Inh concentrate (Immuno-Mycologics, Inc., Norman, OK). Unfortunately, 2 mo postinfusion of this agent, the patient developed chronic non-A, non-B viral hepatitis, documented by characteristic changes on liver biopsy some 8 mo later. In spite of the marked clinical response to prednisolone, the Clq and C4 serum levels and C $\bar{1}$ -Inh functional activity have failed to return to measurable levels. At present the patient remains symptom free on daily therapy of 12 mg prednisolone and 100 mg azathioprine, used as a steroid sparing agent. Now, more than 5 yr postpresentation, no evidence of underlying disease has been detected.

SDS-PAGE

SDS-PAGE using 10% wt/vol acrylamide was performed according to the general method of Laemmli (11). The samples (~ 2 μ g/track) were added to an equal volume of sample buffer (0.125 M Tris, 8 M Urea, 20 mM iodoacetamide, 4% SDS, 20% glycerol, pH 6.8 for unreduced samples; and the same buffer made 10% with 2-mercaptoethanol in place of iodoacetamide for reduced samples) and the samples boiled for 4 min. Silver stain (Bio-Rad Laboratories, Richmond, CA) used for visualization of protein bands was performed according to the manufacturer's instructions. The molecular weight of visible protein bands was determined, by electrophoresis, in the presence of reducing agents, of protein standards of known molecular weight (Sigma Chemical Co., St. Louis, MO).

Isolation and characterization of C $\bar{1}$ -Inh synthesized by patient monocytes

Isolation and culture of patient and normal peripheral blood mononuclear cells. Mononuclear cells were prepared from heparinized peripheral venous blood by Ficoll-Hypaque centrifugation (12). The cells were then washed three times in HBSS and resuspended to 1×10^7 cells/ml in RPMI 1640 containing 20% heat inactivated fetal calf serum. 500 μ l of the cell suspension was added to each well of a 24 well tissue culture plate and the cells incubated for 2 h at 56°C in a humidified atmosphere of 5% CO $_2$. The nonadherent cells were then removed by vigorous washing in RPMI.

Assay of C $\bar{1}$ -Inh secreted in culture

On days 1, 3, 5, and 7 the culture supernatants were removed and replaced by fresh medium. In some cultures, *Escherichia coli* derived recombinant γ -interferon (Immuneron, BG8201, lot No. 10 M05; Biogen, S.A., Geneva, Switzerland) was used to stimulate monocyte secretion of C $\bar{1}$ -Inh (13). C $\bar{1}$ -Inh levels in culture supernatants were measured by an enzyme linked immunoadsorbent assay (14). The cumulative amounts of C $\bar{1}$ -Inh secreted into the culture fluid was found to be linear over the culture period. In order to quantify the

number of cells in culture the monolayers were washed on day 7 and the cells lysed in 200 μ l of SDS (0.05%). The DNA content was measured by spectrofluorimetry (15). 1 μ g of DNA was found to equate to 1×10^5 cells (15). The levels of secreted C $\bar{1}$ -Inh were expressed as molecules per cell per minute.

The functional activity of C $\bar{1}$ -Inh in the culture supernatants was measured by hemolytic assay (16). Results were expressed as Z 1 units/ng C $\bar{1}$ -Inh protein.

Structural characterization of secreted and internalized C $\bar{1}$ -Inh

Internal labeling of monocyte C $\bar{1}$ -Inh was performed using [35 S]-methionine (New England Nuclear, Boston, MA). On the third day of culture the supernatants were removed and the cells were washed with warm (37°C) methionine free Dulbecco's minimum essential medium (DMEM)¹ and the culture continued in DMEM (330 μ l/well at 37°C containing [35 S]methionine (500 μ Ci/ml). After 1 h the supernatants were removed and the monolayers washed and lysed. The supernatants and lysates were centrifuged and stored at -70°C until required.

C $\bar{1}$ -Inh was immunoprecipitated from lysates and supernatants by the addition of 5 μ l of anti-C $\bar{1}$ -Inh diluted 1:10 (15). The immune complexes so formed were isolated by binding to *Staphylococcus aureus* (Sigma Chemical Co.). After centrifugation the bacteria were washed four times in PBS containing Triton X-100 (1%) and the complexes were eluted by the addition of 63.8 mM Tris/3% SDS/5% 2-mercaptoethanol/10% glycerol pH 6.8. The eluates were boiled for 2 min and subjected to SDS-PAGE.

Isolation of C $\bar{1}$ -Inh from plasma of patient and normal individuals

C $\bar{1}$ -Inh was prepared from 150 ml of plasma obtained from the patient and a healthy volunteer using minor modifications of previously published procedures (2, 3). All procedures were performed at 4°C. Fractions were monitored for C $\bar{1}$ -Inh using rocket immunoelectrophoresis with polyclonal rabbit anti human C $\bar{1}$ -Inh (Dako Corp., Copenhagen, Denmark). Briefly, freshly drawn plasma was made 0.01 M in EDTA and benzamidine. Solid PEG 4000 was added all at once to a final concentration of 5% wt/vol and stirred for 1 h. The supernatant was then applied to a lysine sepharose column (Pharmacia Fine Chemicals, Piscataway, NJ) to remove plasminogen. All of the unabsorbed protein was pooled and made 1 mM with PMSF (Sigma), equilibrated with 20 mM sodium phosphate/50 mM NaCl/5 mM EDTA pH 7.0 and applied to a column of DEAE-Sephacel (Pharmacia) equilibrated with the same buffer. C $\bar{1}$ -Inh was eluted using a linear gradient made from starting buffer and the same buffer made 300 mM with NaCl. Fractions containing C $\bar{1}$ -Inh were pooled, made 1 mM with PMSF, dialyzed against 20 mM Tris-HCl/100 mM NaCl, pH 8.0, and applied to a column of concanavalin A-Sepharose (Pharmacia) equilibrated with the same buffer. Elution was achieved with starting buffer made 0.5% wt/vol with α -methylmannoside (Sigma). Ceruloplasmin, found as a contaminant in the C $\bar{1}$ -Inh fractions, was removed by repeating the ion exchange chromatography step using DE 52 (Whatman, Inc., Clifton, NJ) in place of the DEAE-Sephacel. The final C $\bar{1}$ -Inh preparation was dialyzed against 20 mM Tris-HCl/100 mM NaCl pH 8.0. The concentration of the purified C $\bar{1}$ -Inh (normal 0.5 mg/ml, patient 0.5 mg/ml) was determined at 280 nm, assuming an extinction coefficient (1 mg/ml) of 3.6 and molecular weight of 105 kD (2, 3).

Purification of the activated enzymes C $\bar{1}$ s, C $\bar{1}$ r and plasmin and patient anti C $\bar{1}$ -Inh antibody

C $\bar{1}$ s. C $\bar{1}$ s was isolated by elution from immune complexes prepared by mixing at equivalence human albumin (Kabi, Stockholm, Sweden) and rabbit anti-human albumin (Dako) using the method of Arlaud and Colomb (17). Fractions were monitored for C $\bar{1}$ s activity using the substrate *N* α -carbobenzyoxy-*l*-lysine-*p*-nitrophenyl ester (ZLNE) (Sigma). The final preparation was equilibrated against 5 mM triethanolamine-HCl/145 mM NaCl, pH 7.4, and stored at -70°C. This C $\bar{1}$ s

preparation migrated as a single band of apparent molecular weight 83 kD and was fully activated as determined by analysis of reduced and unreduced samples on SDS-PAGE (18). The C \bar{I} s concentration (0.2 mg/ml) was determined at 280 nm assuming an extinction coefficient of 10.5 and molecular weight of 84 kD (19).

C \bar{I} r. C \bar{I} r was also isolated by elution from immune complexes (17). C \bar{I} r was detected in fractions using rocket electrophoresis with goat anti-human C \bar{I} r (Atlantic Antibodies, Scarborough, ME). The final C \bar{I} r preparation was equilibrated with 5 mM triethanolamine-HCl/145 mM NaCl, pH 7.4 and stored at 4°C. The C \bar{I} r preparation migrated on SDS-PAGE as a single band of apparent molecular weight 87 kD and electrophoresis of reduced and unreduced samples demonstrated the preparation to be fully active. C \bar{I} r concentration (0.2 mg/ml) was determined at 280 nm assuming an extinction coefficient of 11.5 and a molecular weight of 84 kD (19). This preparation had no activity against the ZLNE and hence contained no contaminating C \bar{I} s or plasmin.

Plasmin

Plasminogen was prepared from normal plasma using lysine Sepharose affinity chromatography and stored at -70°C (20). Immediately before use, the plasminogen (0.45 mg/ml) was activated using streptokinase (Hoechst) by incubating at 37°C for 15 min using a molar ratio of 200:1 plasminogen to streptokinase (21). The plasminogen was fully activated by this procedure as visualized using SDS-PAGE of reduced and unreduced samples and migrated as a single band of apparent molecular weight 78 kD.

Patient anti C \bar{I} -Inh

Antibody. Details of the isolation and characterization of patient anti-C \bar{I} -Inh antibody have been described previously (10). Antibody concentration (0.8 mg/ml) was measured at 280 nm assuming an extinction coefficient of 13.6 and molecular weight of 150 kD. On SDS-PAGE this antibody migrated as three distinct bands of apparent molecular weight 205–230 kD and a minor band of 150 kD. In the presence of reducing agents the preparation was apparent as two bands of approximate molecular weights 55 and 25 kD representing the heavy and light chains of IgG. Electrophoretic transfer of both reduced and unreduced SDS-PAGE separated antibody to nitrocellulose and subsequent immunofixing with a γ heavy chain specific and with an anti-kappa and anti-lambda antisera (Dako) demonstrated that all of the bands reacted with either anti-heavy chain or anti-kappa antisera but not with anti-lambda. The affinity purified autoantibody is therefore probably monoclonal in nature. However the heterogenous nature of the antibody on SDS-PAGE could be indicative of an antibody of oligoclonal origin, or more probably, may represent immunoglobulin fragments or aggregates generated during its purification. The affinity purified antibody had no enzymatic activity when tested with the substrate ZLNE. The subclass of the antibody (IgG-1) was determined using single radial immune diffusion and monoclonal antibodies to the IgG subclasses 1–4 (Oxoid Ltd., Basinstoke, England).

Studies on patient and normal C \bar{I} -Inh isolated from plasma

The purity and molecular weight of patient and normal C \bar{I} -Inh was assessed using SDS-PAGE. The functional integrity of patient and control C \bar{I} -Inh was assessed by measuring its ability to prevent the colorimetric conversion of the substrate ZLNE by purified C \bar{I} s (2) and also by their ability to form stable complexes, visualized using SDS-PAGE, with the purified enzymes C \bar{I} s, C \bar{I} r, and plasmin. Equimolar quantities of patient (0.5 mg/ml) and normal (0.5 mg/ml) C \bar{I} -Inh were incubated separately at 37°C in 0.02 M sodium phosphate/100 mM NaCl pH 7.2 (PBS) with C \bar{I} s (0.2 mg/ml), C \bar{I} r (0.2 mg/ml) and plasmin (0.45 mg/ml). At the end of 15- and 30-min incubation periods identical aliquots, calculated to contain ~ 1 μ g of both inhibitor and protease, were incubated in sample buffer with and without 2-mercaptoethanol and boiled for 4 min. Samples were then analyzed using SDS-PAGE and the proteins visualized with silver stain.

Studies on the interaction of patient antibody, normal C \bar{I} -Inh, and protease

Normal C \bar{I} -Inh was incubated with affinity purified anti C \bar{I} -Inh antibody in a molar ratio of 1:2 (inhibitor to antibody) at 37°C for 15 min. At the end of this incubation period aliquots of the reaction mixture were removed and incubated separately with each purified protease in a molar ratio of 1:1, inhibitor to protease, at 37°C for 15 and 30 min. The following controls were included: equimolar amounts of native inhibitor and each protease; antibody treated inhibitor and PBS; native inhibitor and PBS; protease with PBS; and affinity purified antibody alone. At the end of the reaction period, aliquots containing the same amounts of C \bar{I} -Inh and protease per aliquot were removed and boiled in sample buffer with and without reducing agents for 4 min.

To study the effect of antibody on C \bar{I} -Inh/protease complexes, C \bar{I} -Inh was preincubated with C \bar{I} s for 15 min and then exposed to antibody and incubated for 15 and 30 min as above.

Kinetic studies on the mechanism of interaction of protease, antibody, and normal C \bar{I} -Inh

To study the mechanism and sequence of reaction between C \bar{I} -Inh, protease and antibody, kinetic studies were performed using the proteases C \bar{I} s, C \bar{I} r, and plasmin. Equimolar quantities of protease and native inhibitor were incubated in PBS, pH 7.2, at 37°C. At the same time, equimolar quantities of antibody treated inhibitor (molar ratio 2:1 antibody to inhibitor) and protease were incubated under the same conditions. Aliquots of these reaction mixtures were withdrawn at time intervals ranging from 10 s to 45 min and immediately analyzed using SDS-PAGE.

Studies on the specificity of affinity purified patient antibody

Affinity purified patient autoantibody was labeled with I¹²⁵ using Enzymobead radioiodination reagent (Bio-Rad Laboratories), according to the manufacturer's instructions. Sephadex G25 (Pharmacia) was used for the separation of bound and free iodine. Patient plasma C \bar{I} -Inh, normal plasma C \bar{I} -Inh, α 1 protease inhibitor (Sigma), antithrombin III (Boehringer Mannheim, Indianapolis, IN), ovalbumin (Sigma) and bovine serum albumin (DiaMed AG, Morat-Murten, Switzerland) were separately linked to cyanogen bromide activated Sepharose at a concentration of 1 mg of protein/ml of packed Sepharose. Greater than 95% binding of each protein to the Sepharose was achieved. Unoccupied sites on the activated Sepharose were blocked using 0.1 M Tris/HCl pH 8. The protein linked Sepharose suspensions were washed four times with 0.1 M phosphate, 0.2 M NaCl pH 7.2 and 400 μ l of a 50% suspension of each protein linked Sepharose was incubated overnight at 4°C with 10 μ l of ¹²⁵I labeled antibody and continuously agitated. The total radioactivity in each tube was then counted and the Sepharose suspensions quickly washed three times (0.1 M phosphate, 0.2 M NaCl, pH 7.2) by centrifugation and the radioactivity remaining bound to the Sepharose measured. These assays were performed in triplicate.

Results

Synthesis of C \bar{I} -Inh by patient monocytes

The intracellular form of C \bar{I} -Inh synthesized by the patient's monocytes was indistinguishable from C \bar{I} -Inh synthesized by normal monocytes when analyzed by SDS-PAGE (Fig. 1). Both normal and patient intracellular forms of C \bar{I} -Inh migrated with an apparent molecular weight of 75 kD. C \bar{I} -Inh secreted by patient monocytes was also indistinguishable from that secreted by normal monocytes and had an apparent molecular weight of 102 kD. The specific functional activities of monocyte secreted C \bar{I} -Inh were 1.4 U/ng protein for the normal control, and 1.53 U/ng for the patient.

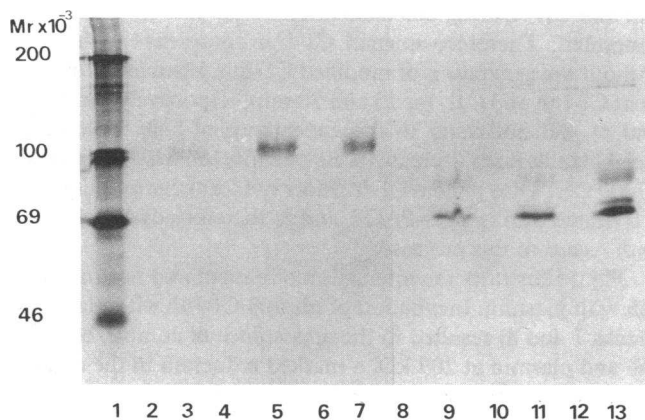


Figure 1. C $\bar{1}$ -Inh synthesized by patient monocytes. Immunoprecipitates of C $\bar{1}$ -Inh from monocyte culture supernatants, (tracks 3, 5, 7) and monocyte lysates tracks 9, 11, and 13 were separated on 10% SDS-PAGE in the presence of reducing agents. Molecular weight markers are in track 1 and tracks 2, 4, 6 are control immunoprecipitates of supernatants and tracks 8, 10, 12 are control immunoprecipitates of lysates using normal rabbit serum in place of anti-C $\bar{1}$ -Inh. Tracks 2, 3, 8, and 9 were obtained using monocytes from a patient with type 1 C $\bar{1}$ -Inh deficiency. Tracks 4, 5, 10, and 11 were obtained using monocytes from a normal control and tracks 6, 7, 12, and 13 represent the patient monocytes. In all three individuals the apparent molecular weight of the intracellular form of C $\bar{1}$ -Inh was 75 kD, while that of the secreted form was 102 kD.

Monocytes from the normal control and from the patient produced C $\bar{1}$ -Inh at similar rates. The synthesis rate for the control monocytes was 332 ± 47 (mean of three cultures \pm SEM) molecules/cell per min, while that for the patient was 375 ± 39 molecules/cell per min. In the presence of recombinant γ -interferon (10 ng/ml) the secretion rates were 2432 ± 241 and 2754 ± 368 molecules/cell per min for the control and patient monocytes respectively.

Patient monocytes therefore secrete, at normal rates, structurally and functionally normal C $\bar{1}$ -Inh.

C $\bar{1}$ -Inh isolated from patient's plasma

C $\bar{1}$ -Inh isolated from normal plasma migrated as a single band on SDS-PAGE with an apparent molecular weight of 115 kD under nonreducing conditions and 105 kD under reducing conditions, (tracks 2 and 3, respectively, Fig. 2). An additional

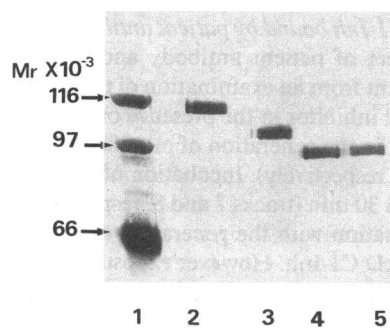


Figure 2. C $\bar{1}$ -Inh from the patient's plasma. C $\bar{1}$ -Inh isolated from normal and patient plasma were separated using 10% SDS-PAGE in the presence (tracks 3, 5) and absence (tracks 2, 4) of reducing agents. Molecular weight markers are in track 1, normal C $\bar{1}$ -Inh in tracks 2 and 3, and patient C $\bar{1}$ -Inh in tracks 4 and 5. On repeated runs the apparent molecular weight of normal C $\bar{1}$ -Inh was 115 and 105 kD (unreduced and reduced, respectively), and patient C $\bar{1}$ -Inh had an apparent molecular weight of 96 kD that was not affected by reducing agents.

band with an apparent molecular weight of 96 kD was also present in trace amounts under nonreducing conditions. The increased mobility of normal C $\bar{1}$ -Inh on SDS gels in the presence of reducing agents has been noted by other workers (22, 23). Patient C $\bar{1}$ -Inh isolated from plasma also migrated as a single band on SDS-PAGE but in contrast had an apparent molecular weight of 96 kD under both reducing and non reducing conditions (tracks 4 and 5, respectively [Fig. 2]). Thus C $\bar{1}$ -Inh isolated from patient's plasma had a lower apparent molecular weight than normal C $\bar{1}$ -Inh, irrespective of the presence or absence of reducing agents.

Functional activity of patient plasma C $\bar{1}$ -Inh

Patient inhibitor isolated from plasma had no inhibitory activity against C $\bar{1}$ s as assessed using the esterolytic assay. The functional activity of the patient's C $\bar{1}$ -Inh was also examined by measuring its ability to form SDS stable complexes with the purified proteases electrophoresed in the absence of reducing agents. These experiments are illustrated in Fig. 3–5, tracks 1–8. In Fig. 3 the protease C $\bar{1}$ s was used; in Figs. 4 and 5, C $\bar{1}$ r and plasmin, respectively, were employed. The format for each of these three experiments is essentially the same and is as follows: patient inhibitor incubated in PBS, pH 7.2, at 37°C for 30 min is illustrated in track 1; normal inhibitor under the same conditions, track 5; protease in PBS, tracks 2 and 6; patient inhibitor incubated for 15 and 30 min with protease, tracks 3 and 4, respectively; and normal inhibitor and protease incubated under the same conditions, tracks 7 and 8, respectively.

Reaction of patient and normal C $\bar{1}$ -Inh with C $\bar{1}$ s

Reaction of patient and normal C $\bar{1}$ -Inh with C $\bar{1}$ s is illustrated in Fig. 3, tracks 1–8. Examination of tracks 7 and 8, which show the effect of incubation of normal C $\bar{1}$ -Inh and C $\bar{1}$ s for 15 and 30 min, respectively, demonstrates a marked decrease in the quantity of native 115 kD inhibitor, a decrease in C $\bar{1}$ s (83 kD), and the appearance of a new high molecular weight band at 225 representing complexed C $\bar{1}$ s and C $\bar{1}$ -Inh. A slight increase in the quantity of modified C $\bar{1}$ -Inh at 96 kD is

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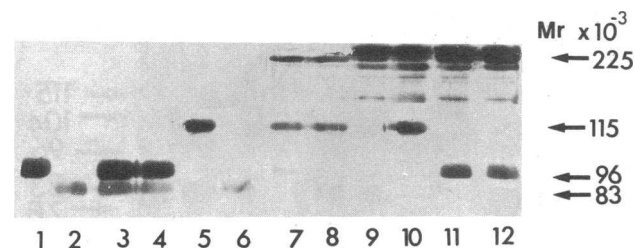


Figure 3. Interaction of C $\bar{1}$ -Inh purified from patient and normal plasma with C $\bar{1}$ s and autoantibody. Patient C $\bar{1}$ -Inh incubated for 30 min at 37°C in PBS is illustrated in track 1; normal inhibitor also incubated for 30 min at 37°C in track 5; protease incubated under the same conditions, tracks 2 and 6; patient inhibitor incubated for 15 and 30 min with C $\bar{1}$ s, tracks 3 and 4, respectively; and normal inhibitor incubated with C $\bar{1}$ s under the same conditions, tracks 7 and 8, respectively; affinity purified autoantibody incubated in PBS for 30 min, track 9; affinity purified autoantibody incubated with normal C $\bar{1}$ -Inh for 30 min in track 10; and affinity purified autoantibody preincubated with normal C $\bar{1}$ -Inh for 15 min and then incubated with C $\bar{1}$ s for 15 and 30 min are in tracks 11 and 12, respectively. The proteins were separated with 10% SDS-PAGE in the absence of reducing agents. The apparent molecular weight of each species is indicated with an arrow.

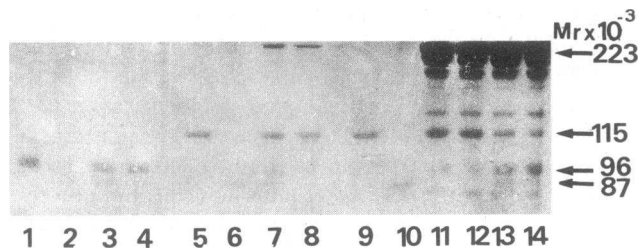


Figure 4. Interaction of C $\bar{1}$ -Inh purified from patient and normal plasma with C $\bar{1}$ r and autoantibody. Patient C $\bar{1}$ -Inh incubated for 30 min at 37°C in PBS is illustrated in tracks 1; C $\bar{1}$ r incubated under the same conditions, tracks 2 and 6; patient C $\bar{1}$ -Inh incubated with C $\bar{1}$ r for 15 and 30 min, tracks 3 and 4; normal C $\bar{1}$ -Inh incubated with PBS for 30 min at 37°C in tracks 5 and 9; normal C $\bar{1}$ -Inh incubated with C $\bar{1}$ r for 15 and 30 min, tracks 7 and 8, respectively; affinity purified patient autoantibody and normal C $\bar{1}$ -Inh incubated together for 15 and 30 min, tracks 11 and 12, respectively; normal C $\bar{1}$ -Inh pretreated with affinity purified patient autoantibody and then incubated with C $\bar{1}$ r for 15 and 30 min, tracks 13 and 14, respectively. Proteins were separated using 10% SDS-PAGE in the absence of reducing agents.

also apparent. Therefore under these conditions normal C $\bar{1}$ -Inh complexes with C $\bar{1}$ s with the generation of a small amount of modified 96 kD C $\bar{1}$ -Inh. Patient C $\bar{1}$ -Inh treated in an identical manner (tracks 3 and 4) does not form stable complexes with, nor is it altered by C $\bar{1}$ s and is therefore dysfunctional with regard to this protease.

Fig. 4, tracks 1–8, illustrates the reaction of patient and normal C $\bar{1}$ -Inh with C $\bar{1}$ r. It is evident from tracks 7 and 8 that incubation of normal C $\bar{1}$ -Inh with C $\bar{1}$ r results in reduction in the quantity of native inhibitor present at 115 kD, reduction in the quantity of C $\bar{1}$ r at 87 kD and the appearance of a high molecular weight band at 223 kD representing complexed C $\bar{1}$ -

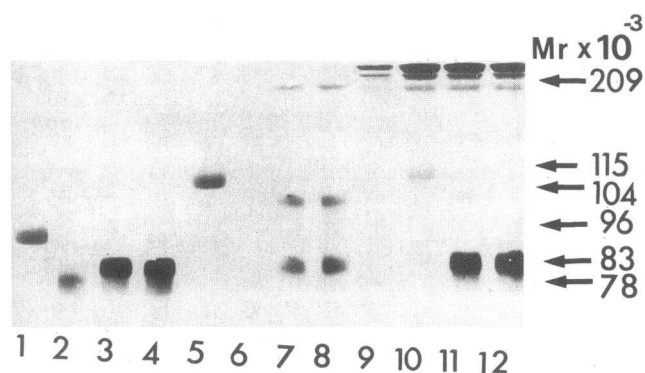


Figure 5. Interaction of C $\bar{1}$ -Inh purified from patient and normal plasma with plasmin and autoantibody. Patient C $\bar{1}$ -Inh incubated in PBS for 30 min is illustrated in track 1; plasmin in track 2; patient C $\bar{1}$ -Inh and plasmin incubated for 15 and 30 min at 37°C tracks 3 and 4, respectively; normal C $\bar{1}$ -Inh incubated for 30 min in PBS track 5; track 6 contains no protein; normal C $\bar{1}$ -Inh and plasmin incubated for 15 and 30 min tracks 7 and 8, respectively; affinity purified autoantibody track 9; affinity purified autoantibody and normal C $\bar{1}$ -Inh incubated for 30 min track 10 (normal C $\bar{1}$ -Inh staining poorly in this track); affinity purified autoantibody preincubated with normal C $\bar{1}$ -Inh for 15 min and then exposed to plasmin for 15 and 30 min tracks 11 and 12, respectively. The proteins were separated in 10% SDS-PAGE in the absence of reducing agents.

Inh and C $\bar{1}$ r. During this incubation 96 kD inhibitor was not generated. Therefore normal C $\bar{1}$ -Inh complexes with C $\bar{1}$ r without the generation of modified C $\bar{1}$ -Inh. Incubation of patient C $\bar{1}$ -Inh and C $\bar{1}$ r, for 15 and 30 min, respectively (tracks 3 and 4), did not result in the appearance of high molecular weight bands nor a decrease in the quantity of 96 kD C $\bar{1}$ -Inh or 87 kD C $\bar{1}$ r. Thus patient C $\bar{1}$ -Inh does not form complexes with C $\bar{1}$ r stable during SDS-PAGE and is therefore dysfunctional with regard to this protease.

Fig. 5 illustrates the interaction of patient and normal C $\bar{1}$ -Inh with plasmin. Incubation of normal C $\bar{1}$ -Inh with plasmin (tracks 7 and 8) resulted in the appearance of complexed C $\bar{1}$ -Inh and plasmin at 209 kD, a marked reduction in the quantity of native C $\bar{1}$ -Inh at 115 kD, the appearance of a new band at ~ 104 kD, a trace amount of inhibitor appearing at 96 kD and a new C $\bar{1}$ -Inh species apparent at 83 kD. Thus normal C $\bar{1}$ -Inh forms complexes with plasmin and is also converted to a 104- and 83-kD species. Similar findings regarding the interaction of C $\bar{1}$ -Inh and plasmin have been reported by other workers (21, 24, 25). In contrast, interaction of patient C $\bar{1}$ -Inh with plasmin (tracks 3 and 4), resulted in disappearance of inhibitor at 96 kD with a new major C $\bar{1}$ -Inh band appearing at 83 kD without the generation of stable high molecular weight complexes. Since patient C $\bar{1}$ -Inh does not complex with plasmin, it is dysfunctional with regard to this protease. However, patient C $\bar{1}$ -Inh is rapidly cleaved by plasmin to an 83-kD species.

The above findings may be summarized as follows: although patient monocytes secrete structurally and functionally normal C $\bar{1}$ -Inh, this protein circulates in the patient's plasma in an inactive 96-kD form. Patient's C $\bar{1}$ -Inh is therefore modified in vivo postsynthesis and secretion. It is well documented that interaction of normal C $\bar{1}$ -Inh with protease can under certain circumstances result in the generation of modified and inactive 96 kD C $\bar{1}$ -Inh (21, 24–26). Using the assay systems we have described, inactivation of C $\bar{1}$ r by C $\bar{1}$ -Inh does not produce modified inactive C $\bar{1}$ -Inh, inactivation of C $\bar{1}$ s generates a small quantity of 96-kD inhibitor and inactivation of plasmin produces modified 104- and 83-kD inhibitor. To investigate the role of this patient's autoantibody in the generation of 96 kD from normal C $\bar{1}$ -Inh, normal C $\bar{1}$ -Inh was preincubated with patient autoantibody and this antibody-treated C $\bar{1}$ -Inh was reacted with, in separate experiments, each of the proteases: C $\bar{1}$ s, C $\bar{1}$ r, and plasmin.

Interaction of normal C $\bar{1}$ -Inh, patient antibody, and protease

Effect of C $\bar{1}$ s on normal C $\bar{1}$ -Inh bound by patient antibody. Fig. 3 also illustrates the effect of patient antibody and C $\bar{1}$ s on normal C $\bar{1}$ -Inh. It is evident from an examination of this figure that incubation of normal inhibitor in the presence or absence of antibody did not result in the generation of modified 96 kD C $\bar{1}$ -Inh, (tracks 10 and 5, respectively). Incubation of C $\bar{1}$ s and normal C $\bar{1}$ -Inh for 15 and 30 min (tracks 7 and 8, respectively) resulted in complex formation with the generation of a trace quantity of modified 96 kD C $\bar{1}$ -Inh. However exposure of antibody treated C $\bar{1}$ -Inh to C $\bar{1}$ s for 15 and 30 min (tracks 11 and 12) resulted in the disappearance of 115 kD inhibitor and a pronounced increase in the quantity of inhibitor present at 96 kD.

To examine directly whether or not complex formation had occurred between C $\bar{1}$ s and antibody treated C $\bar{1}$ -Inh the

same samples were analyzed by SDS-PAGE in the presence of reducing agents. The results of this experiment are illustrated in Fig. 6. Complexes of C \bar{I} -Inh and the light chain of C \bar{I} s appeared at 130 kD (tracks 1 and 2). It is apparent however that in the presence of antibody (tracks 5, 6), only a trace quantity of complexes are found. These results clearly show that when normal C \bar{I} -Inh bound by patient antibody is exposed to C \bar{I} s, there is enhanced cleavage of the normal C \bar{I} -Inh to a 96-kD protein with a marked reduction in the quantity of complexes observed. If normal C \bar{I} -Inh is preincubated with C \bar{I} s and then exposed to antibody, cleavage of C \bar{I} -Inh does not take place (results not illustrated). This indicates, therefore, that once complex formation has taken place, patient antibody does not affect normal C \bar{I} -Inh.

Effect of C \bar{I} r on normal C \bar{I} -Inh bound by patient antibody.

The same experimental procedure was used for investigating the effect of C \bar{I} r on antibody treated normal C \bar{I} -Inh and the results are illustrated in Fig. 4. It is clear from tracks 7 and 8 and 11 and 12, respectively, that interaction of normal inhibitor with C \bar{I} r or with antibody did not produce significant amounts of modified 96 kD inhibitor. Exposure of antibody treated C \bar{I} -Inh to C \bar{I} r (tracks 13 and 14) resulted in a marked increase in the quantity of 96 kD C \bar{I} -Inh. On reduction of these same samples only a trace quantity of the C \bar{I} -Inh/C \bar{I} r complex was visible in the presence of antibody (data not illustrated). It is concluded that C \bar{I} r, as is the case with C \bar{I} s, mediates cleavage of C \bar{I} -Inh to which antibody has bound.

Effect of plasmin on normal C \bar{I} -Inh bound by patient antibody. Fig. 5 illustrates the interaction of antibody bound normal C \bar{I} -Inh and plasmin. The experiment follows the same outline as that used for C \bar{I} s and C \bar{I} r. Incubation of normal C \bar{I} -Inh and plasmin (tracks 7, 8) resulted in the appearance of an inhibitor/plasmin complex at 209 kD, the absence of inhibitor present at 115 kD and an increase in the amount of inhibitor staining at 104 and 83 kD. Exposure of antibody treated C \bar{I} -Inh to plasmin (tracks 11 and 12) results in all of the inhibitor appearing as an 83-kD species. This 83-kD C \bar{I} -Inh is the same as the species produced by cleavage of patient C \bar{I} -Inh by plasmin (tracks 3, 4). Thus plasmin interaction with normal C \bar{I} -Inh bound by patient antibody results in an altered cleavage pattern of normal C \bar{I} -Inh with all of the inhibitor appearing as an 83-kD species. The basis of this altered cleavage is discussed in a later section.

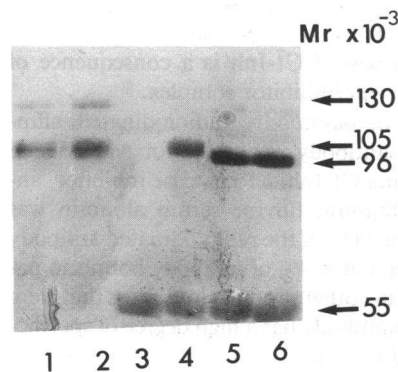


Figure 6. Interaction of normal C \bar{I} -Inh, patient autoantibody and C \bar{I} s. These proteins were separated using 10% SDS-PAGE in the presence of 5% 2-mercaptoethanol. Normal C \bar{I} -Inh incubated with C \bar{I} s for 15 and 30 min is illustrated in tracks 1 and 2, respectively; affinity purified autoantibody track 3; affinity purified autoantibody and normal

C \bar{I} -Inh incubated for 30 min track 4; affinity purified autoantibody preincubated with normal C \bar{I} -Inh for 15 min and then further incubated with C \bar{I} s tracks 5 and 6, respectively.

It is demonstrated that normal C \bar{I} -Inh treated with patient antibody and further incubated with the proteases C \bar{I} s and C \bar{I} r, results in enhanced cleavage of normal C \bar{I} -Inh to a 96-kD protein and in the case of plasmin results in enhanced cleavage to an 83-kD protein. These experiments also demonstrated that this effect cannot be mediated by antibody alone. This enhanced cleavage of C \bar{I} -Inh by each of the proteases tested, could occur according to two distinct mechanisms. Patient antibody could bind to C \bar{I} -Inh altering the tertiary structure of the molecule in such a way as to generate a new site and allow direct cleavage of the inhibitor by each of the proteases. Alternatively antibody could bind to C \bar{I} -Inh in a manner that did not interfere with normal interaction between C \bar{I} -Inh and its target proteases but which renders the acyl intermediate thought to develop between inhibitor and protease unstable (25, 26). This unstable complex might then dissociate into active enzyme and modified inhibitor. If patient antibody was mediating cleavage of C \bar{I} -Inh by enhancing the dissociation of inhibitor/protease complexes, then it could be predicted that complex formation would be the rate limiting step in the cleavage of C \bar{I} -Inh bound by patient antibody. A kinetic study was therefore performed to investigate the mechanism of cleavage of C \bar{I} -Inh by autoantibody and protease.

Studies on the reactive mechanism of protease cleavage of normal C \bar{I} -Inh bound by patient antibody

Equimolar quantities of normal C \bar{I} -Inh and C \bar{I} s, C \bar{I} r or plasmin were incubated in PBS, pH 7.2, at 37°C and aliquots removed at varying time intervals from 10 s to 30 min. At the same time equimolar quantities (inhibitor to protease) of C \bar{I} -Inh bound by patient antibody was incubated with each protease and aliquots removed at the same time intervals. The aliquots were then immediately boiled in sample buffer for 4 min and analyzed using SDS-PAGE. When normal C \bar{I} -Inh was incubated with C \bar{I} s, complex formation took place in less than 10 s in the absence of antibody. Using antibody-bound C \bar{I} -Inh, cleavage of normal C \bar{I} -Inh to a 96-kD protein also took place in less than 10 s, making interpretation of the sequence of the reaction difficult. However, if the same experiment was performed at 8°C, using 30-s time intervals it was observed that complex formation between C \bar{I} -Inh and C \bar{I} s occurred at 3 min and cleaved modified inhibitor appeared at 4 min. Similarly, when the reaction between C \bar{I} r and C \bar{I} -Inh and autoantibody was monitored at 37°C in the presence and absence of antibody it was observed that complex formation occurred at 4 min and cleaved C \bar{I} -Inh also appeared at the same time interval.

The sequence of reaction of normal C \bar{I} -Inh with plasmin is illustrated in Fig. 7, tracks 1-9. It is evident from these tracks that 104 kD inhibitor was formed from 115 kD inhibitor within 10 s of incubation (track 1). Visible complexes (209 kD) appear between C \bar{I} -Inh and plasmin when an incubation period of 2 min had elapsed (track 3). In the following tracks (with a time span of 2-45 min) there was a reduction in the quantity of 115 kD inhibitor, an initial increase in the quantity of 104 kD C \bar{I} -Inh followed by a diminution of this species and a small amount of 83 kD C \bar{I} -Inh generated throughout the reaction period. The reaction between C \bar{I} -Inh bound by patient antibody and plasmin is shown in tracks 10-18. It can be observed that there was a progressive decrease throughout the incubation period in the quantity of native 115 kD C \bar{I} -Inh. It can also be seen that 104 kD C \bar{I} -Inh was present in track 10,

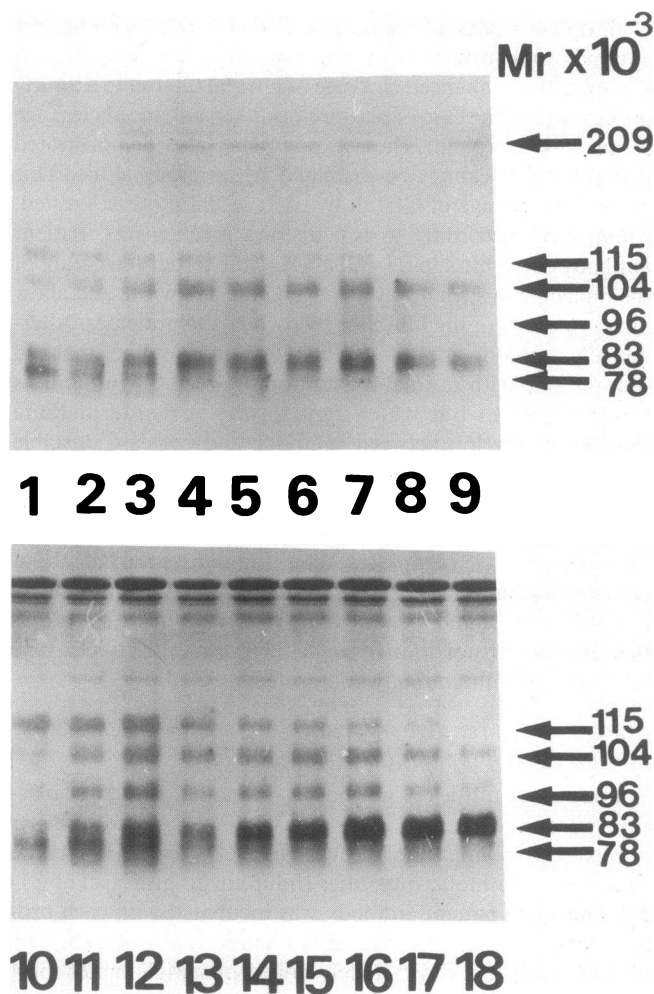


Figure 7. Kinetic study of the interaction of normal C $\bar{1}$ -Inh, patient autoantibody and plasmin. Normal C $\bar{1}$ -Inh and plasmin were incubated at 37°C and aliquots were removed at time intervals of 10 s to 45 min and separated using 10% SDS-PAGE in the absence of reducing agents, tracks 1–9. Normal C $\bar{1}$ -Inh pretreated with patient autoantibody was also incubated under the same conditions with plasmin, aliquots were removed at the same time intervals and analyzed on the same gel, tracks 10–18. Tracks 1, 10 are the proteins incubated for a period of 10 s; tracks 2, 11 for 60 s; tracks 3, 12 for 2 min; tracks 4, 13 for 3 min; tracks 5, 14 for 4 min; tracks 6, 15 for 5 min; tracks 7, 16 for 15 min; tracks 8, 17 for 30 min; tracks 9, 18 for 45 min.

that this increased in quantity from track 10 to 16 (10 s to 15 min) and thereafter showed a decrease to the end of the incubation period. 96 kD C $\bar{1}$ -Inh is apparent in track 11 and also decreases in quantity at the end of the reaction period. After an incubation period of 4 min (track 14), a sharp increase in the quantity of 83 kD C $\bar{1}$ -Inh was apparent that increased in quantity to the end of the reaction period.

It is evident therefore from each of these experiments that the formation of cleaved C $\bar{1}$ -Inh in the presence of antibody was occurring after complexes first appeared between normal C $\bar{1}$ -Inh and enzyme in the absence of antibody and consequently they are likely to be closely related events.

As can be seen from Fig. 7 in the absence of antibody normal C $\bar{1}$ -Inh complexes with plasmin and 104- and 83-kD C $\bar{1}$ -Inh species are generated. In the presence of autoantibody,

however, a new species of C $\bar{1}$ -Inh at 96 kD is apparent which is followed by a sharp increase in the presence of 83 kD C $\bar{1}$ -Inh. This 96-kD species produced by plasmin in the presence of patient autoantibody is the same molecular weight species as patient C $\bar{1}$ -Inh, which as already shown is rapidly cleaved by plasmin to an 83-kD species. Furthermore, in a separate experiment we incubated normal C $\bar{1}$ -Inh with plasmin over various time intervals, terminated the reaction with soya bean trypsin inhibitor, and then further incubated with C $\bar{1}$ s and have demonstrated that the species of cleaved C $\bar{1}$ -Inh produced by plasmin in the absence of antibody is functionally active (results not illustrated).

The interaction between plasmin and C $\bar{1}$ -Inh is therefore more complicated than that which occurs between C $\bar{1}$ -Inh and other proteases. Harpel and Cooper have previously described the generation of two species of 96 kD C $\bar{1}$ -Inh during its encounter with plasmin, one of which retained functional activity (24). A further cleavage product of C $\bar{1}$ -Inh of 85 kD may also be generated by plasmin (25). In our initial experiments two species of C $\bar{1}$ -Inh were generated by plasmin and autoantibody of ~ 96 kD molecular weight. Refinements in the running conditions of the SDS-PAGE have allowed us to separate these two species at 104 and 96 kD.

To explain these observations on the interaction of plasmin and C $\bar{1}$ -Inh in the presence and absence of antibody, the following reaction mechanisms are proposed. In the absence of patient antibody, C $\bar{1}$ -Inh is directly cleaved by plasmin to a 104-kD species that is fully active. This species is not further cleaved by plasmin. After a time lapse, stable complex formation between functionally active forms of C $\bar{1}$ -Inh and plasmin takes place. In the presence of patient antibody the sequence of reaction is initially identical, and C $\bar{1}$ -Inh is directly cleaved by plasmin to a functionally active 104-kD species. After the same time lapse complex formation between C $\bar{1}$ -Inh bound by patient antibody and plasmin takes place. However due to the presence of patient antibody these complexes are unstable and immediately break down, releasing functionally inactive 96-kD C $\bar{1}$ -Inh. This inactive C $\bar{1}$ -Inh (like patient C $\bar{1}$ -Inh) is then cleaved by plasmin to an 83-kD protein. Thus patient antibody facilitates cleavage of normal C $\bar{1}$ -Inh to a 96-kD protein with each of the proteases we have tested. This 96-kD C $\bar{1}$ -Inh is the same molecular weight species of C $\bar{1}$ -Inh found circulating in the patient's plasma. The cleavage of normal C $\bar{1}$ -Inh to a 96-kD protein in the presence of antibody is associated with a decrease in the amount of complexes formed and furthermore is apparently rate limited by complex formation between C $\bar{1}$ -Inh and protease. It is therefore likely that the antibody facilitated cleavage of C $\bar{1}$ -Inh is a consequence of destabilization of the enzyme/inhibitor complex.

Specificity of patient autoantibody. Radioiodinated, affinity purified, patient autoantibody was tested for reactivity to normal and patient plasma C $\bar{1}$ -Inh, α 1 protease inhibitor, antithrombin III and ovalbumin. Bovine serum albumin was used as a negative control. 90% of the radioiodinated antibody bound to normal C $\bar{1}$ -Inh and < 4% of antibody bound to patient C $\bar{1}$ -Inh and each of the other protease inhibitors that were tested. Thus patient autoantibody has a high degree of specificity for normal uncleaved C $\bar{1}$ -Inh.

Discussion

C $\bar{1}$ -Inh is a member of an important class of inhibitors known as the α 1 proteinase inhibitors (also called serpins), which cir-

culate in high levels in human plasma (2–4, 27–31). It is thought, based largely on information derived from studies involving SDS-PAGE, that inhibitors of this class rapidly inactivate target proteases by forming tightly bound equimolar inhibitor/protease complexes. It has been observed in vitro that during the interaction of C $\bar{1}$ -Inh with target protease, modified inactive inhibitor may be generated with an apparent molecular weight lower than that observed for native inhibitor (4, 22, 23, 32).

Under the conditions described in this report normal C $\bar{1}$ -Inh migrates with an apparent molecular weight of 115 kD in the absence of reducing agents and 105 kD in the presence of reducing agents. It was also demonstrated that normal C $\bar{1}$ -Inh may be converted to a 96-kD species following interaction with protease and autoantibody and furthermore, that the molecular weight of this species was not altered by the presence of reducing agents. Patient plasma C $\bar{1}$ -Inh also migrated with a molecular weight of 96 kD irrespective of the presence or absence of reducing agents. Therefore both patient plasma C $\bar{1}$ -Inh and normal C $\bar{1}$ -Inh modified by protease and autoantibody are distinct from the 105-kD C $\bar{1}$ -Inh produced from normal C $\bar{1}$ -Inh by the use of reducing agents. Thus, differences in mobility between reduced normal C $\bar{1}$ -Inh and reduced C $\bar{1}$ -Inh after interaction with protease can best be explained by a difference in the primary structures (i.e., molecular size) of the two species. Hence, it is apparent that the structural basis of the generation of modified C $\bar{1}$ -Inh during encounter with protease does not simply involve an alteration in the secondary structure of the molecule and is almost certainly the result of proteolytic cleavage and release of a small peptide of ~ 4 kD molecular weight from the parent molecule. This cleavage of the C $\bar{1}$ -Inh molecule probably occurs at the arginine-threonine residues, 444–445 as numbered by Bock et al. (29).

It has been shown that patient monocytes secrete structurally and functionally normal C $\bar{1}$ -Inh. It was also demonstrated however, that this protein circulates in plasma in an inactive, structurally altered form with an apparent molecular weight of 96 kD. It has previously been observed in vitro that 96 kD C $\bar{1}$ -Inh may be generated from 105 kD C $\bar{1}$ -Inh during encounter with protease. Furthermore, Zuraw and Curd have demonstrated that this occurs to a limited extent in vivo as part of the normal catabolism of C $\bar{1}$ -Inh since small quantities of modified C $\bar{1}$ -Inh were found circulating in the plasma of normal individuals (25). Therefore, it is highly probably that the 96-kD C $\bar{1}$ -Inh found circulating in this patient's plasma has been produced in vivo by protease interaction with the normal species of C $\bar{1}$ -Inh secreted by the patient's monocytes and hepatocytes. As all of the patient's C $\bar{1}$ -Inh circulates as a 96-kD species, a highly efficient in vivo mechanism must exist for the conversion of the normal species of C $\bar{1}$ -Inh to the structurally modified, inactive form. The presence of an autoantibody in the patient's circulation which bound to normal C $\bar{1}$ -Inh, suggested that an autoimmune mechanism was responsible for the generation of this dysfunctional C $\bar{1}$ -Inh. In this report, experiments are described that investigate the generation of 96 kD C $\bar{1}$ -Inh from normal (105 kD) C $\bar{1}$ -Inh following inhibitor, protease, and autoantibody interaction. It was found that patient autoantibody promoted cleavage of normal C $\bar{1}$ -Inh to a 96-kD species with each of the proteases that were investigated: C $\bar{1}$ r, C $\bar{1}$ s, and plasmin.

The precise mechanism by which autoantibody mediated

this effect was also investigated and it was shown that patient antibody greatly diminished the quantity of stable complexes formed during C $\bar{1}$ -Inh protease interaction. Furthermore, it was demonstrated kinetically, that autoantibody facilitated cleavage of C $\bar{1}$ -Inh was occurring at the same time or subsequent to complex formation between the inhibitor and protease. Hence, patient autoantibody apparently mediates its effect by binding to normal C $\bar{1}$ -Inh, altering its conformation and consequently causing destabilization of the acyl intermediate normally formed between protease and inhibitor. The demonstration that patient autoantibody facilitated cleavage of normal C $\bar{1}$ -Inh by each of the target proteases we have tested is a novel observation, at the molecular level, on the pathogenesis of autoimmune disease.

The patient's autoantibody was shown to react with normal uncleaved C $\bar{1}$ -Inh but not with patient's cleaved C $\bar{1}$ -Inh. Furthermore autoantibody had no effect on C $\bar{1}$ -Inh if it was added after C $\bar{1}$ -Inh/protease interaction. That the antibody has a highly restricted specificity is further illustrated by its failure to react with the other members of the serpin family that have been tested. Support for the suggestion that the three dimensional structure of modified inactive C $\bar{1}$ -Inh is altered from that of the native molecule is provided by Nuijens et al. (33) who prepared a monoclonal antibody reactive with modified inactive C $\bar{1}$ -Inh but not with the native molecule.

Since our initial report (and during the preparation of this manuscript), five other cases of angioedema with anti C $\bar{1}$ -Inh antibodies have been described (34, 35). The authors of one of these reports suggest that C $\bar{1}$ s is solely responsible for the inactivation of C $\bar{1}$ -Inh in the presence of patient autoantibody (34). In contrast to our studies, these authors utilized unfractionated serum or plasma to study cleavage products of radio-labeled C $\bar{1}$ -Inh. In contrast to the autoantibody we have described, one of the autoantibodies described by Alsenz et al. reacts with patient C $\bar{1}$ -Inh (34). It is therefore probable that a spectrum of autoantibodies exists in autoimmune angioedema having unique specificities for sites on C $\bar{1}$ -Inh. This could result in preferential cleavage of C $\bar{1}$ -Inh by different proteases with each particular autoantibody. Our studies clearly show that our patient's autoantibody mediates cleavage of normal C $\bar{1}$ -Inh with each of the proteases we have tested.

The antibody we have described is apparently monoclonal in nature and is specific for a site close to or at the reactive site of normal C $\bar{1}$ -Inh. There is some evidence that the anti C $\bar{1}$ -Inh antibodies described in some of the other cases of autoimmune angioedema may also be monoclonal or oligoclonal in nature (34). Monoclonal immunoglobulins have also been observed in association with the previously describe form of acquired angioedema (9). There is therefore a striking association between all forms of acquired angioedema and monoclonal gammopathy. Furthermore C $\bar{1}$ -Inh has been shown to circulate as an 96-kD species in acquired angioedema (25) and these patients also have depressed Clq. At this stage it is unclear whether or not there is a closer relationship between autoimmune angioedema and this more common form of acquired angioedema, which is thought to be associated with antiidiotypic antibodies (36).

Kallikrein and activated Hageman factor in particular have also been implicated in the pathogenesis of angioedema and modified C $\bar{1}$ -Inh may be produced on interaction with these proteases (22, 25, 37–39). Therefore C $\bar{1}$ -Inh may have a com-

mon mechanism of inactivation with all of its target proteases and consequently in vivo, the autoantibody may facilitate destabilization of each of the acyl intermediates formed during each protease/C1-Inh interaction.

Autoimmune angioedema is therefore a new autoimmune disease the biochemical hallmarks of which are low serum Clq and C4 levels, and absent functional C1-Inh activity in the presence of normal or slightly reduced serum antigenic levels. As in most cases of autoimmune disease, it is unclear what has induced the production of anti C1-Inh antibody. Complement activation after an infection, for example, may have exposed a site on C1-Inh in the vicinity of the reactive site that is not normally accessible to the immune response.

The normal remittive agents that were used in the treatment of angioedema produced only a transient clinical response in this patient's case. However, treatment with agents of proven value in modulation of autoimmune disorders has induced a lasting clinical remission. This remission has occurred without a change in the level of circulating antibody (data not included) or a recovery in the serum levels of C4, Clq, or functional C1-Inh activity. That remission has been induced by this therapy is supported by the recurrence of the edema on the three separate occasions when attempts were made to reduce prednisolone dosage. The precise mechanism by which this therapy has mediated the cessation of attacks of edema is unknown.

In summary, we have described the mechanism by which an autoantibody to C1-Inh, found in a patient with autoimmune angioedema, mediates C1-Inh dysfunction. These findings contribute to our understanding of the mechanisms of interaction between C1-Inh and proteases. They also represent novel observations on pathogenic mechanisms in autoimmune disease and have important implications in the management of complement-mediated angioedema.

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